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INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

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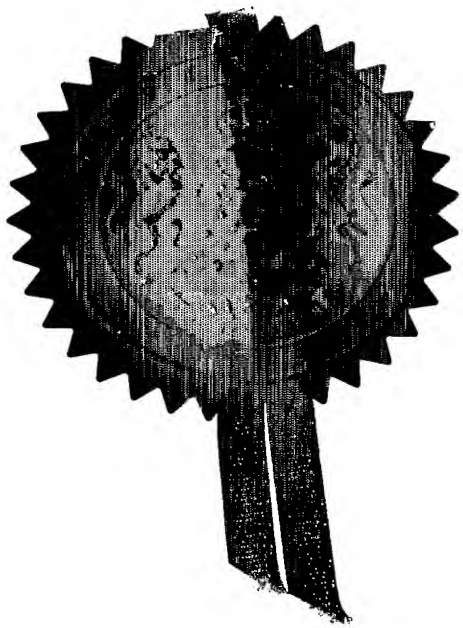

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Request for grant of a patent

The Patent Office
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South Wales NP10 8QQ

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2. Patent Application Number
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Sphere Medical Limited
Harston Mill
Harston
Cambridgeshire
CB2 5GG

8606295001

Patents ADP number *(if known)*

If the applicant is a corporate body, give the country/state of its incorporation

Country: **England**
State:

4. Title of the invention

Chemical Sensor

5. Name of agent
"Address for Service" in the United Kingdom to which all correspondence should be sent

Beresford & Co
16 High Holborn
London WC1V 6BX

Patents ADP number

1826001

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications filed in the last 12 months.

Country	Priority application number	Date of filing

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BERESFORD & Co

12. Name and daytime telephone number of person to contact in the United Kingdom

MACDOUGALL; Alan John Shaw

Tel: 020 7831 2290

Patent application

Chemical sensor

Peter G. Laitenberger, Stuart P. Hendry, Gavin L. Troughton

Introduction

Chemical sensors, in particular biosensors have found widespread use in numerous applications. In order to create a sensor for a specific analyte these sensors typically consist of a chemical recognition element, e.g. a chemical receptor, which allows a molecule, or class of molecules, to be identified, coupled to a means of signal transduction. The presence of the analyte of interest causes a measurable change in a physical property of transduction material. A wide range of transduction modalities to convert the physico-chemical response to the analyte in the test medium into the measurement signal have been developed. Examples include amperometric, potentiometric, conductimetric, optical, gravimetric, surface-acoustic waves (SAW), thermal or capacitive principles.

To make any transducer specifically respond to a particular analyte some kind of recognition event needs to occur at the transducer's surface. The recognition elements used in chemical or biosensors have traditionally consisted of either

- Chemically selective membranes, for the measurement of simple molecules and ions, such as dissolved gasses, electrolytes and pH; or
- Biomolecules, such as proteins (enzymes, antibodies), nucleic acids (DNA or RNA) or even whole microorganisms for more complex analytes, in particular biologically derived species.

While the transduction methods are generally well developed and can be manufactured in a straight-forward manner, the recognition elements employed in chemical sensors lag far behind. These recognition elements are generally of poor reproducibility and fragile, seriously limiting the operating and storage conditions of the sensors and greatly reducing their operational or storage lifetime. Moreover, the development and deployment costs of such sensors are generally high. Scientists have therefore tried to develop alternatives to these traditionally used recognition elements.

A promising route to overcome these issues is offered by synthetic receptors, for example, molecularly imprinted polymers (MIP). Molecular imprinting is the process of template-induced formation of specific recognition sites in a material where the template, i.e. the analyte of interest or a close analogue, directs the orientation and binding of the material's structural components by a self-assembly process. It consists of the following key steps:

1. Functional monomers are allowed to interact reversibly with a template molecule in solution.
2. The hereby formed template assemblies are copolymerised with a cross-linking monomer resulting in a cross-linked network polymer.
3. The template is displaced and the resulting MIP-material can be used for selective molecular recognition of the corresponding compound.

MIP are a generic and cost-effective technique for preparing synthetic receptors and therefore promise to overcome the drawbacks suffered by the currently used receptors in chemical sensors.

MIP have so far been mainly prepared in the form of continuous blocks that need to be crushed and sieved before use. While the resulting particles find widespread use in chromatographic separation their applicability to chemical sensors is rather limited. For applications in chemical sensors, MIP are preferably used in the form of films. These films may advantageously be produced by in-situ polymerisation, from a liquid mixture comprising the molecule which serves as a template, one (or more) polymerisable or polycondensable functional monomer(s), one or more crosslinking agent(s) and one (or more) polymerisable initiator(s). This mixture may then be deposited by spotting, centrifugation, screen printing, spin coating or by any means capable of obtaining, after evaporation of any possible solvent, a uniform deposition of the reagents. The polymerisation may be carried out in a variety of ways, e.g. thermally, chemically, electrochemically or photochemically.

While a range of MIP-based chemical sensors have been demonstrated based on a range of transduction principles (e.g. resistive, amperometric, SAW, gravimetric, optical and potentiometric), they suffer from two significant drawbacks:

1) The advent of microsystems technology has provided the capability to create small chemical sensors and the ability to integrate several chemical sensors on the same substrate. The functionalisation of these sensor elements requires the deposition of a number of (potentially) different MIP-coatings on the sensor elements in close proximity to one another. To date, the MIP-deposition techniques are very limited with respect to the minimum distances which can be achieved between sensor elements covered by different MIP-layers. Moreover, the deposition of different MIP-layers on the same substrate typically involves elaborate procedures, in which each layer is deposited and locally polymerised one after the other. Local polymerisation can for example be achieved by exposing the polymerisable coating to spatially modulated radiation (e.g. light) through a photomask.

2) The affinity of the MIP for the analyte of interest and its selectivity can vary widely depending on a range of factors. It is generally assumed that a change in selectivity of molecularly imprinted polymers in comparison to non-imprinted polymers can occur only when the analyte has an increased number of interaction points with the MIP. In some cases, the interaction of analytes and components of the test medium (e.g. solvent in the case of liquid samples) with non-imprinted parts of a polymer may overshadow the interactions of the analyte with the true imprint so that the selectivity is not as good as required for the application,

irrespective of the imprinting efficacy. The number of binding sites present in the polymer that play a role in the selective interaction of the analyte with the imprint (cavity) is typically less than 1% but can amount to 35% of the theoretical maximum amount of binding sites[1]. A schematic representation of the selective and the non-selective interactions is given in Figure 1.

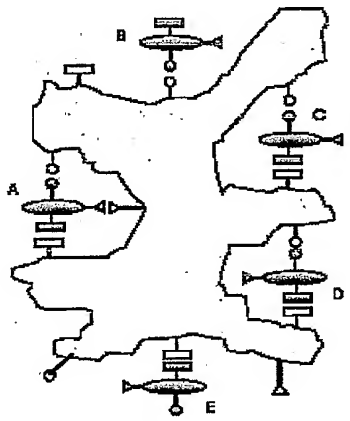

Figure 1: Schematic representation of the interaction of the analyte with the molecularly imprinted polymer which contains several binding sites. The most favourable energetic interaction is at location A, where three binding sites result in the most selective interaction between the analyte and the MIP. Two-point interactions, which are energetically less favourable and therefore less selective, are depicted at locations C and D. Locations B and E show the least selective interaction possible.

The invention

The invention presented in this document addresses the shortfalls identified above by providing a straight-forward means of functionalising chemical sensors with appropriate recognition elements, for example synthetic receptors and MIP. It is particularly applicable, but not limited to micromachined chemical sensors. One particular example of a silicon-based microsensor chip with multiple chemical sensor elements is shown in Figure 2.

One preferred embodiment of the invention comprises the creation of structures on the device substrate around one or more features, e.g. sensor element(s), which act to contain the mixture of some or all of the reagents which will be used to create the synthetic receptor(s) or MIP(s) employed as the chemical recognition element(s) of the device or sensor. Due to the containment within the structure a larger number of functionalised sensor elements can be created in a given surface area on the substrate. Moreover, different mixtures can coexist on the surface of the substrate at the same time without mixing or cross-contamination. In addition, the confinement

[1] K.J. Shea, D.A. Spivak and B. Sellergren, "Polymer components to nucleotide bases. Selective binding of adenine derivatives to imprinted polymers, J. Am. Chem. Soc. 115, 3368-3369 (1993).

structures provide a means of achieving a uniform or complete coverage of the feature or sensor element even if parts of the mixture (e.g. a solvent) evaporate.

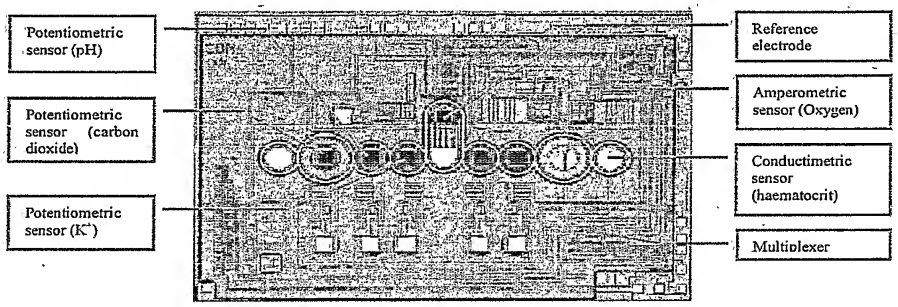

Figure 2: Example of a multi-parameter chemical sensor chip developed by Sphere Medical Ltd.

The structures may be pool-like or of any general shape which will be suited to the application in hand. In general, the shape of the structures will be chosen to suit the size and shape of the feature(s) or sensor element(s) and also to maximise the number of features/sensor elements in a given surface area. The sensor elements may for example employ electrochemical (e.g. potentiometric, in particular ISFETs (ion-sensitive field effect transistors) or amperometric), conductimetric, optical, gravimetric, surface-acoustic waves, resonant or thermal principles.

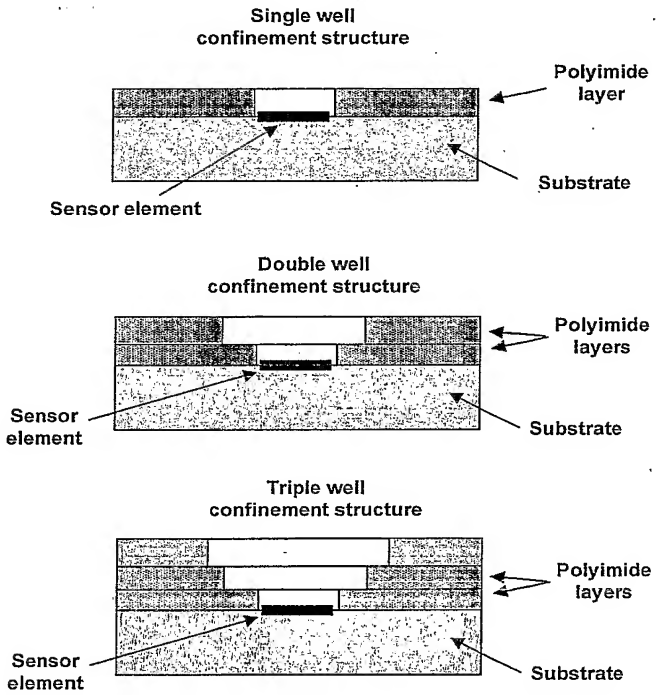

Figure 3: Schematic illustration of different confinement structures which can be created on the substrate. These illustrations serve to demonstrate the principle only and the invention described in this document is not limited to these designs.

Confinement structures may be made of resists (for example, but not limited to, (poly)imide, SU8 etc.), a passivation layer used in the device fabrication (e.g. a silicon nitride, oxide or oxynitride layer) or any other material which is deposited during or after the manufacturing process of the device or which is compatible with the manufacturing process or use of the device, including but not limited to isolators, such as oxides, nitrides, glass, polymers or ceramics, metals or semiconductors. Of particular advantage are materials which can react with the deposited materials in such a way as to improve the adhesion of the receptor layer on the sensor element or substrate. Improved adhesion can improve the lifetime and reliability of the sensor. In addition, features, e.g. mechanical or chemical structures, may be included into the design of the confinement structures which provide for keying between the receptor layer and confinement structure in order to enhance the adhesion of the receptor layer on the substrate.

The confinement structures can be created in one or more processing steps. For example, in one particular process the innermost confinement structures are created by patterning a previously deposited polyimide layer in a suitable manner. The deposition and patterning process steps can be repeated until the desired confinement structures are realised. In another embodiment, the whole confinement structure (single or multiple wells) is created in one patterning step. Also, the confinement structures may be created by building up suitable materials on the substrate. Alternatively, the wells may be created by removing materials from the substrate or layers of materials deposited on the substrate. There are many other approaches which can be used to create these structures which are known to those skilled in the art.

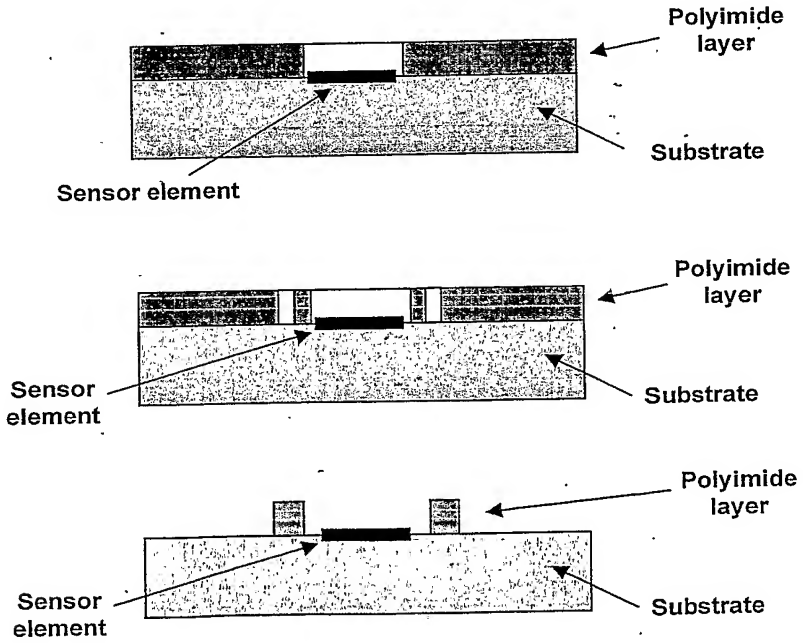

Figure 4: Schematic representation of three examples of single-well designs which may be created on the substrate. These drawings are for illustration only.

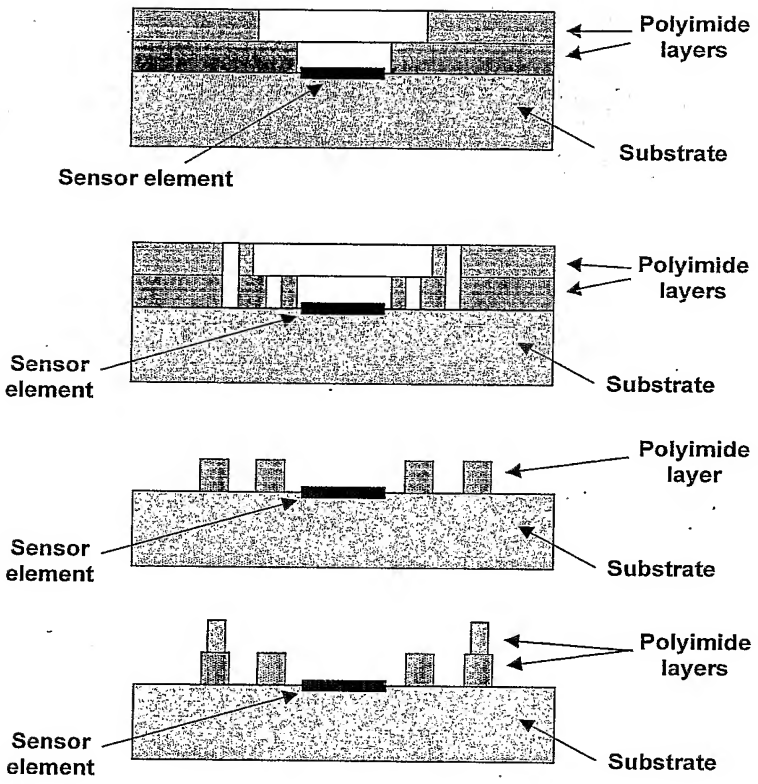

Figure 5: Schematic representation of four examples of double-well designs which may be created on the substrate. These drawings are for the purpose of illustration only.

The depth, size, area, volume and shape of the wells can be chosen to suit the particular application in mind.

The invention described in this document is particularly applicable to, but not limited to, sensors similar to that shown in Figure 2, where two or more sensor elements are located in close proximity on the same substrate.

The structures can consist of single or multiple containment structures nested one inside the other. The confinement structure(s) create(s) one or multiple well(s) on the substrate into which different materials can be deposited. Multiple structures are of particular advantage if the functionalisation of the sensor elements requires the deposition of multiple substances over the sensor element, e.g. the electrolyte of an electrochemical sensor and a chemically-selective membrane. Alternatively, this multiple pool structure can be used to deposit several reagents which will react with one another to result in the creation of the recognition element. Furthermore, multiple containment structures, for example in the form of, but not limited to, rings, can be used to deposit membranes which, for example, aid the containment of earlier deposited materials during the lifetime of the sensor. Alternatively these membranes may, for example, act as filter or recognition elements themselves.

In a preferred embodiment, one or more confinement structures are created around one or multiple sensor element(s). These structures are then filled with one or more mixtures comprising, for example, the molecule which serves as a template, one (or more) polymerisable or polycondensable functional monomer(s), one or more crosslinking agent(s) and one (or more) polymerisable initiator(s). Different mixtures can be filled into different wells. Once the required number of structures is filled, the polymerisation of the mixtures is carried out, using, for example but not limited to, thermal, chemical or photochemical initiation. Once the polymerisation is completed, the template molecule is removed or displaced from the polymerised material(s) using, e.g. dissolution in one or more appropriate solvent(s). The remaining material(s) can then serve as chemical recognition element(s) to provide selective molecular recognition of the corresponding compound(s) of interest.

The main advantage of this approach in contrast to methods currently employed is its speed and simplicity. Polymerisation of different MIP can occur in parallel rather than serially. Moreover, different polymerisable mixtures (each in their own well) can coexist on the substrate without mixing and cross-contamination.

The molecule which serves as a template may typically be an ion, an organic molecule of biological or synthetic origin, a protein, polypeptide, a polynucleotide, a polysaccharide, a medical drug or any other chemical species, bacterium, virus, protozoa or micro-organism which may be of value in detecting selectively.

Rather than adding all components of the polymerisation mixture in one deposition step, individual components of the mixture can be deposited one at a time in order to create mixtures of different compositions in different wells on the substrate.

In another embodiment, the same (e.g. polymer) matrix materials are used and deposited into one or more confinement structures on the substrate. In a prior or subsequent deposition one or more template molecule(s) and/or one or more functional monomer(s) are added to the respective confinement structures, typically one type of template molecule per well. After polymerisation, the template molecule(s) are removed in one or more dissolution steps, leaving behind different functional chemical receptor layers in each confinement structure.

It may also be advantageous to add the polymeriser first in order to promote polymerisation close to substrate, which may aid adhesion of the recognition element to the substrate.

In order to get rid of spurious effects associated, for example, with temperature fluctuations, it is generally advantageous to combine two identical transducer devices, only one of which is coated with a sensitive layer, and to carry out differential measurements. Using the present invention, an additional advantage may be obtained by carrying out a differential measurement on two transducers that are identical except for the fact that one is coated with a molecularly imprinted material and the other is coated with a material of identical composition, polymerised and/or crosslinked in the absence of the template molecule (see Figure 8 for a schematic illustration of one particular embodiment of this aspect of the invention). The reason for this is that a MIP

Deposition of materials into the wells created by the confinement structures can be carried out in a number of ways, which include, but are not limited to, ink-jet printing, microspotting, dripping, pipetting, droplet transfer (using e.g. a needle structure) etc. Single and multiple dispensing heads may be used in order to enable serial or parallel deposition into the wells. An example of such a deposition process is shown in Figure 6.

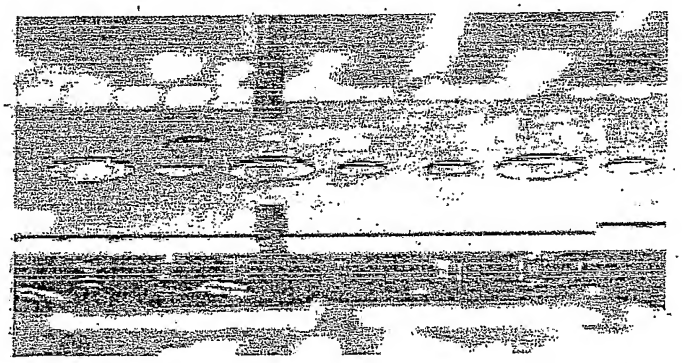

Figure 6: Deposition of materials into confinement structures around sensor elements on the chip shown in Figure 2 using a microspotter.

In some cases it may be advantageous to dilute the mixture or create a suspension to be deposited by the addition of solvents, for example, to enable the deposition of particles in the form of a suspension or to adjust the viscosity of the liquid to be deposited. Once deposited, solvents may evaporate. In such cases, it may be desirable to deposit a larger volume in the confinement structures than is ultimately required for the operation of the sensor element.

In addition to simple confinement of the mixture by the well(s) on the substrate surface tension and hydrophobic/hydrophilic interactions may be employed to aid the confinement. This may be particularly advantageous in situations, where a larger volume has to be deposited into the wells than can accommodated in the wells themselves, e.g. due to evaporation or any other form of volume loss during the manufacture, storage or life of the device.

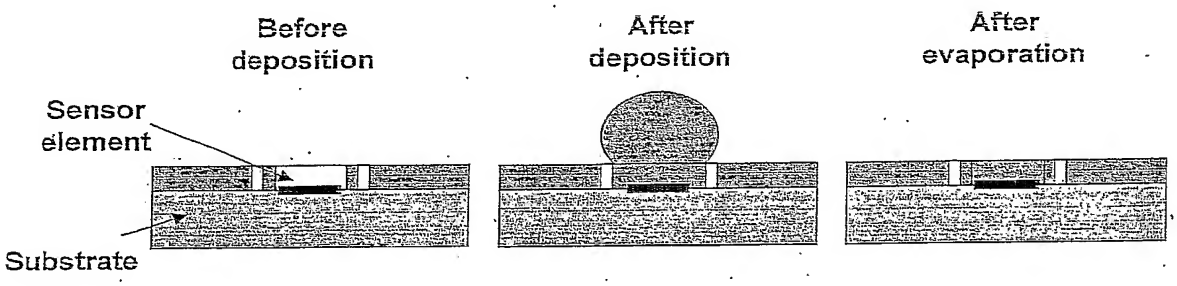

Figure 7: Illustration of the containment of the mixture in a well in a situation where part of the mixture may evaporate. In order to provide adequate filling of the well an excess volume of the mixture is deposited. This excess volume is contained by the confinement structure using surface tension effects. After evaporation of the excess volume the well is adequately filled.

material can have, besides the binding sites specifically suited to the analyte to be detected, non-specific sites which can bind other molecules. On the other hand, the material polymerised in the absence of the template possesses only non-specific sites. It is thus possible to compensate either fully or partially for the interference which may be due to molecules other than the analyte, which become bound to the sensitive layer by non-specific interactions. In the present invention, the two transducers can be combined on the same substrate by creating confinement structures around the individual sensor elements and realising the template-imprinted MIP and the material crosslinked in the absence of a template in the confinement structures around the two respective sensing elements.

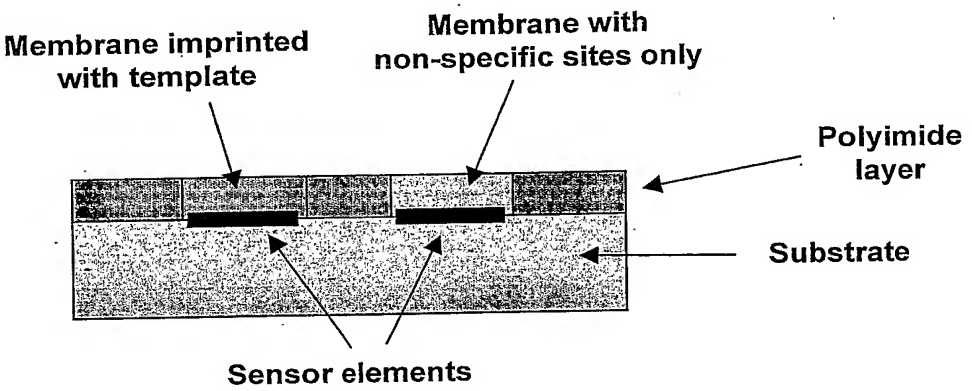

Figure 8: Example of a substrate with two identical sensing elements, one of which is functionalised using a MIP imprinted with the analyte of interest, while the other is functionalised with a membrane of identical composition, but polymerised in absence of the template. This second sensing element serves as a reference sensor to identify and account for non-specific interactions of the analyte and test medium with the MIP-membrane.

Rather than providing a reference transducer with a receptor material which was crosslinked in the absence of a template molecule, further embodiments of the invention employ one or more reference sensor(s) functionalised with a receptor material sensitive to any of the following species or any combination thereof:

- One or more interfering species to the analyte(s) of interest;
- One or more products of chemical reactions involving the analyte(s) of interest, e.g. a metabolite;
- Derivatives of the analyte(s) of interest;
- Any other chemical species which may affect the sensor operation.

In the simplest embodiment, the signal from the reference sensor may be subtracted from that of the sensor element which is functionalised to the analyte of interest. However, more elaborate compensation schemes may be employed, known to those skilled in the art.

In another embodiment a reference sensor may be created by polymerising the respective mixture in the presence of the target analyte or an analogue and then using this receptor material without dissolving or removing the analyte or analogue from the polymerised material. In this material all or many of the specific recognition sites are occupied by the analyte or analogue, while the non-specific site can still provide binding sites for molecules and other structures (e.g. viurs, bacteria micro-organism, etc) other than the analyte of interest. This reference sensor can therefore be used to monitor and compensate (either partially or fully) for non-specific interactions.

In order to promote adhesion of the deposited layer to the substrate, sensor element, confinement structure or electrode, the said surface(s) may be coated beforehand with a layer which allows specific bonds to be created with the recognition elements. In particular, in order to enhance adhesion of the MIP material, the surface of the substrate, confinement structure or transducer may advantageously be functionalised prior to the deposition, the aim of the operation being, for example, to allow, or to promote, the establishment of covalent bonds between the atoms at the surface of the substrate, confinement structure or transducer and the molecules of the coating.

In addition to the synthesis of MIP-structures directly on the chip, the confinement structures may also be used to deposit one or more previously prepared MIP-material(s), for example, but not limited to, in the form of particles, onto the substrate. Additionally these receptor materials may be enclosed in or below a membrane structure, which is deposited into the same or an associated confinement structure (see for example, Figure 9 and Figure 10).

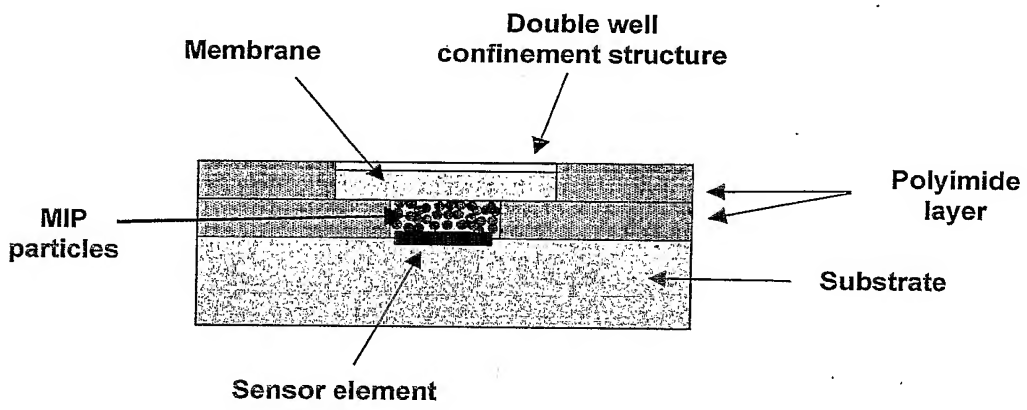

Figure 9: Example of an embodiment of the invention whereby MIP particles are trapped inside a well below a membrane.

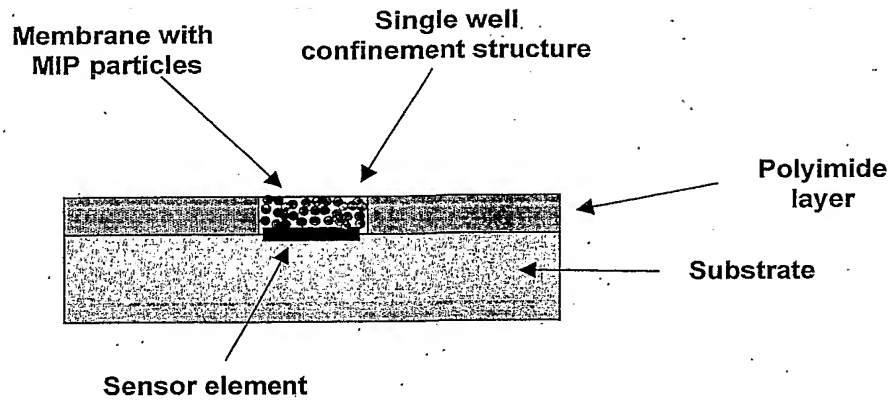

Figure 10: Example of an embodiment of the invention whereby MIP particles are trapped inside a membrane deposited on the sensor element. The single well configuration is shown for illustration only.

In some cases, further purification and concentration of the analyte(s) of interest can be achieved *in situ* by encapsulating or covering the sensing elements in one or more material(s), solid or liquid, into which the analyte(s) of interest preferentially partition(s) over the test medium it is in. One particular example, is an analyte which is in a polar test medium, but which partitions preferentially into a non-polar solvent. The material(s) may be deposited into confinement structures associated with the sensing element(s) for the analyte(s) of interest. An illustrative example of this embodiment is shown in Figure 11. Different confinement structures may contain different materials. It is therefore possible to create different environments, e.g. polar and non-polar, on the same substrate.

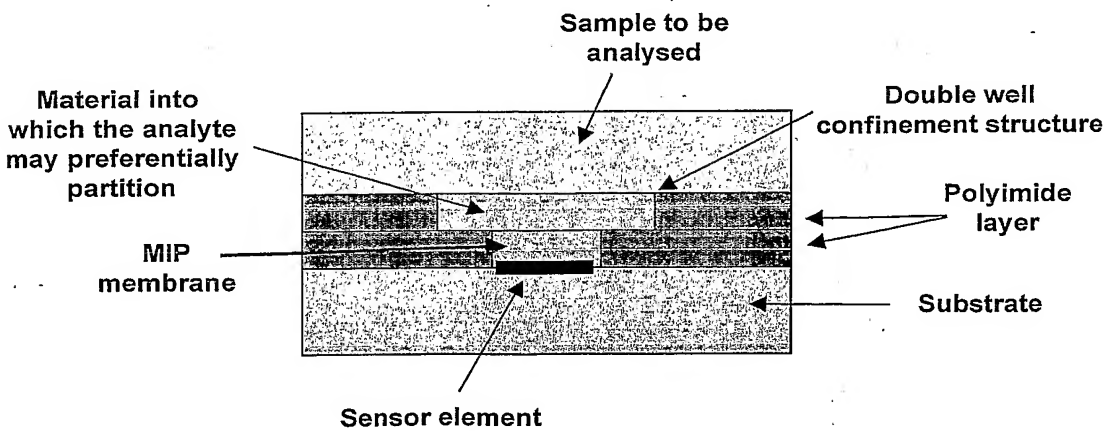

Figure 11: Schematic representation of an example of one embodiment of the invention where a material is deposited on top of a sensor element into which the analyte of interest preferentially partitions.

This approach may be particularly advantageous for the detection of substances which exist as emulsions in polar solvent. One particular example may be certain medical drugs, for example anaesthetics, such as propofol.

In addition to providing preferential partitioning of analyte(s) into structures and materials associated with particular sensing elements, this approach may also provide a specific or desirable environment around a sensing element or receptor, e.g. to improve its performance. For example, many MIP have been designed or optimised for operation in non-polar media. By locally providing a non-polar environment on the chip around the receptor these MIP may be employed in a chemical sensor operating in polar solvents for analytes which will partition into the non-polar material.

A further embodiment of the invention employs a differential measurement between two sensing elements with the same chemical recognition elements, one of which uses a partitioning material, while the second element is fabricated without the partitioning material.

While most of the processes and methods described in this document are illustrated using thermal, chemical, electrochemical or photochemical activation for the polymerisation steps, they are nevertheless compatible with all known polymerisation techniques, as well as evaporation, condensation and freezing.

Also, MIP materials represent only on class of recognition element which can be used in conjunction with the invention. In this document, MIP are used for the purpose of illustration. Other materials, in particular other synthetic receptors, may be employed instead or in addition to MIP.

I this document, the term ion-sensitive field effect transistor or ISFET should also refer to chemically modified field effect transistor, CHEMFET, or more generally any device where the input is a chemical reaction or the presence of a particular chemical in close proximity to the field effect device.